

We claim:

- 1           1.       A microfluidic device comprising:  
2           a main flow path comprising a detection zone and an outlet; and  
3           at least two inlet flow paths wherein the inlet flow paths intersect and merge  
4           into the main flow path at or upstream of the detection zone at an upstream angle of  
5           less than 90°.
- 1           2.       The microfluidic device of Claim 1 further comprising two inlet flow  
2           paths.
- 1           3.       The microfluidic device of Claim 1 further comprising three inlet flow  
2           paths.
- 1           4.       The microfluidic device of Claim 3 wherein the main flow path has at  
2           least one detection zone at or downstream of each intersection of each inlet flow path  
3           with the main flow path.
- 1           5.       The microfluidic device of Claim 1 wherein the main flow path is from  
2           about 0.1  $\mu\text{m}$  deep by 0.1  $\mu\text{m}$  wide to about 1 mm deep by 2 mm wide.
- 1           6.       The microfluidic device of Claim 1 wherein the first inlet flow path is  
2           from about 0.1  $\mu\text{m}$  deep by 0.1  $\mu\text{m}$  wide to about 1 mm deep by 2 mm wide.
- 1           7.       The microfluidic device of Claim 1 further comprising means for  
2           applying a flow inducing force.
- 1           8.       The microfluidic device of Claim 6 wherein the flow inducing force is  
2           electricity.

1        9.        The microfluidic device of Claim 6 wherein the flow inducing force is  
2        negative or positive fluid pressure.

1        10.       The microfluidic device of Claim 9 wherein positive or negative  
2        pressure is applied to the outlet.

1        11.       The microfluidic device of Claim 1 wherein the device further  
2        comprises cells in at least one of the inlet flow paths and the main flow path.

1        12.       The microfluidic device of Claim 1 wherein the device further  
2        comprises leukocytes, a calcium dye and a candidate compound in the main flow path.

1        13.       An observation device comprising a plurality of microfluidic devices of  
2        Claim 1 sharing a common detection zone.

1        14.       The observation device of Claim 13, wherein the main flow paths of  
2        the microfluidic devices are substantially parallel at the common detection zone.

1        15.       An observation device comprising a plurality of microfluidic devices of  
2        Claim 1 wherein the main flow paths of the microfluidic devices are substantially  
3        parallel at their detection zones.

1        16.       A method of observing the effect of a candidate compound on cells in a  
2        microfluidic device comprising:

3        (a) providing a microfluidic device comprising a main flow path comprising a  
4        detection zone, and an outlet and at least two inlet flow paths intersecting and merging  
5        with the main flow path at or upstream of the detection zone;

6        (b) applying at least one cell to a first inlet flow path and the candidate  
7        compound to a second inlet flow path;

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- 8       (c) inducing flow of the cells and the candidate compound toward the outlet;  
9       (d) allowing the cells to mix with the candidate compound at the intersection of  
10      the second inlet flow path and the main flow path; and  
11       (f) observing the effect of the candidate compound on the cells in the detection  
12      zone.

1           17.     The method of Claim 16 wherein the microfluidic device has three inlet  
2      flow paths and a second candidate compound is added to the third inlet flow path.

1           18.     The method of Claim 16 further comprising stopping the flow of the  
2      cells while the cells are in the detection zone.

1           19.     The method of Claim 17 further comprising observing the cells in each  
2      of a number of detection zones wherein the main flow path comprises a plurality of  
3      detection zones, wherein each detection zone is at or downstream of each intersection  
4      of each inlet flow path with the main flow path.

1           20.     The method of Claim 16 wherein the candidate compound being studied  
2      is a cell activator and the cell is a lymphocyte.

1           21.     The method of Claim 17 wherein cells are added to a first inlet flow  
2      path, cell activator is added to a second inlet flow path, and a candidate compound is  
3      added to a third inlet flow path.

1           22.     The method of Claim 21 wherein the candidate compound being studied  
2      is an inhibitor, and the cells are lymphocytes.

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1           <sup>3</sup> 23.   The method of Claim ~~16~~<sup>1</sup> wherein the flow paths are coated with a  
2   substance selected from the group consisting of proteins, glycoproteins, phospholipids,  
3   hydrophilic polymers and hydrophobic polymers.

1           <sup>4</sup> 24.   The method of Claim ~~23~~<sup>I</sup> wherein the flow path is coated with protein.

1           <sup>5</sup> 25.   The method of Claim ~~23~~<sup>3</sup> wherein the flow is induced by an electric  
2   force.

1           <sup>6</sup> 26.   The method of Claim ~~24~~<sup>4</sup> wherein the flow is induced by positive or  
2   negative fluid pressure.

1           27.   A method for studying calcium influx in a lymphocyte comprising:

2           (a) providing a microfluidic device comprising a main flow path having a  
3   detection zone, at least two inlet flow paths sequentially intersecting with the main  
4   flow path upstream of the detection zone and an outlet downstream from the detection  
5   zone;

6           (b) applying lymphocytes to a first inlet flow path and an activator to a second  
7   inlet flow path;

8           (c) inducing flow of the lymphocytes and the activator toward the outlet;

9           (d) allowing the lymphocytes to mix with the activator at the intersection of the  
10   second inlet flow path and the main flow path; and

11          (e) observing the effect of the activator on the lymphocytes in the detection  
12   zone.

1           28.   The method of Claim 27 wherein the microfluidic device comprises  
2   three inlet flow paths further comprising adding a candidate compound to a third inlet  
3   flow path; and observing the effect of the candidate compound on the lymphocytes in  
4   the detection zone.

1 29. A method for studying leukocyte rolling comprising:

2 (a) providing a microfluidic device comprising a main flow path comprising a  
3 detection zone and an outlet and at least two inlet flow paths sequentially intersecting  
4 and merging with the main flow path at or upstream of the detection zone and wherein  
5 the walls of the main flow path in the detection zone have attached thereto cell  
6 adhesion molecules;

7 (b) applying at least one leukocyte to a first inlet flow path;

8 (c) applying a candidate compound to a second inlet flow path;

9 (d) inducing flow of the cells and the compound into the main flow path;

10 (d) allowing the leukocytes, candidate compound and cell adhesion molecules  
11 to interact; and

12 (e) observing the leukocyte rolling in the detection zone.

1 30. The method of Claim 29 wherein the device comprises three inlet flow  
2 paths, further comprising adding an inhibitor to said third inlet flow path; mixing the  
3 inhibitor, leukocytes, candidate compound and cell adhesion molecules and observing  
4 the leukocyte rolling in the detection zone.

1 31. The method of Claim 30 further comprising stopping the flow of the  
2 cells, candidate compound and inhibitor during the mixing step.

1 32. The device of Claim 1 further comprising variations in the cross-section  
2 of the main flow path.

1 33. The device of Claim 32 wherein the variations create a cell trapping  
2 zone.

1 34. The device of Claim 33 wherein said variations are weirs.

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